Wide-field Fast-scanning Photoacoustic Microscopy of Brain Functions in Action

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ABSTRACT: We have developed fast functional photoacoustic microscopy for 3D high-resolution high-speed imaging of the mouse brain. In particular, a novel single-wavelength pulse-width-based method can image blood oxygenation with capillary-level resolution at 100 kHz frame rate.

OCIS codes: (110.5120) Photoacoustic imaging; (180.0180) Microscopy; (220.4000) Microstructure fabrication

INTRODUCTION

High-performance functional brain imaging is extremely important to both fundamental neuroscience and clinical neurology. As a major implementation of photoacoustic tomography, photoacoustic microscopy (PAM) has been proven capable of anatomical, chemical, functional and metabolic imaging [1-4]. Here, we present fast functional PAM, which is capable of high-resolution high-speed imaging of the mouse brain through an intact skull *in vivo* [5]. This PAM technology achieves a lateral spatial resolution of ~3 μm for structural imaging. Using a single-wavelength pulse-width-based method, PAM allows three-dimensional (3D) blood oxygenation imaging with capillary-level resolution at a one-dimensional (1D) imaging rate of 100 kHz. PAM's blood oxygenation imaging speed is 100 times higher than with our acoustic-resolution system [6], and more than 500 times higher than with phosphorescence-lifetime-based two-photon microscopy (TPM) [7].

METHODS

As shown in **Figure 1a**, a pulsed laser beam at 532 nm is focused by an optical objective lens. A beam combiner provides acoustic-optical coaxial alignment. The focused laser beam and the generated photoacoustic waves are both directed by a MEMS scanning mirror plate. Volumetric imaging is provided by fast angular scanning of the MEMS mirror along the *x*-axis and slow linear step-motor scanning of the sample along the *y*-axis (**Fig. 1b**). A 2.4 μm diffraction-limited lateral resolution and 26 μm axial resolution have been achieved, with a maximum penetration depth of 0.8 mm in biological tissue (**Fig. 1c**). The maximum in-focus scanning range is ~3.0 mm along the *x*-axis, with a cross-sectional frame rate of 500 Hz.

By using a single-wavelength pulse-width-based method (PW-sO_2), our PAM system can perform high-speed imaging of the oxygen saturation of hemoglobin (sO_2) (**Fig. 1d**). The two forms of hemoglobin, oxy- and deoxy-hemoglobin (HbO_2 and HbR), have different saturation intensities, defined as the excitation intensity that reduces the absorption coefficient to half its initial value [8]. PW-sO_2 does not suffer wavelength-dependent optical attenuation as the traditional wavelength-tuning method does.

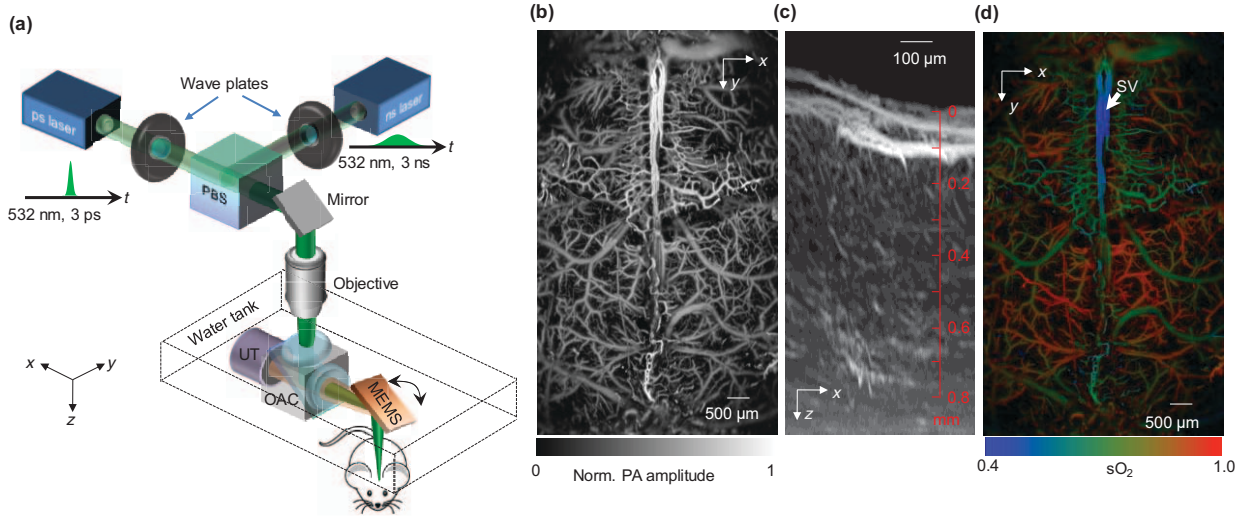

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Fig. 1. Fast functional photoacoustic microscopy (PAM) of the mouse brain. (**a**) Schematic of the PAM system. OAC, optical-acoustic combiner; PBS, polarizing beam splitter; UT, ultrasonic transducer. (**b**) A representative *x*-*y* projected brain vasculature image through an intact skull. (**c**) A representative enhanced *x*-*z* projected brain vasculature image acquired over a 0.6×0.6 mm^2 region with depth scanning, where the signal amplitude was normalized depth-wise. (**d**) PAM of oxygen saturation of hemoglobin (sO_2) in the same mouse brain as (b), acquired by using the single-wavelength pulse-width-based method (PW-sO_2) with two lasers. SV, skull vessel.

RESULTS

Here, as a demonstration, PAM was used to image cortical responses in the mouse brain evoked by electrical stimulation with a volumetric frame rate of 1 Hz. **Figure 2a** shows a PAM image of the mouse brain, where the dense cortical vasculature can be clearly observed. Upon right paw stimulation, the relative PA amplitude change in the somatosensory region of the left hemisphere started to increase and peaked at the end of the stimulation. Upon paw stimulation, the relative PA amplitude change in the somatosensory region of the contralateral hemisphere started to increase and peaked at the end of the stimulation, with a core responding region 0.15 mm beneath the cortical surface (**Fig. 2b**). The increase in PA signal amplitudes reflected the elevated neural activity evoked by the stimulations. The brain responses in the ipsilateral hemispheres consistently showed a similar trend to that of the contralateral hemispheres (**Fig. 2c**), but a weaker magnitude and a longer time lag. This observation may indicate cross talk between the two hemispheres of the brain.

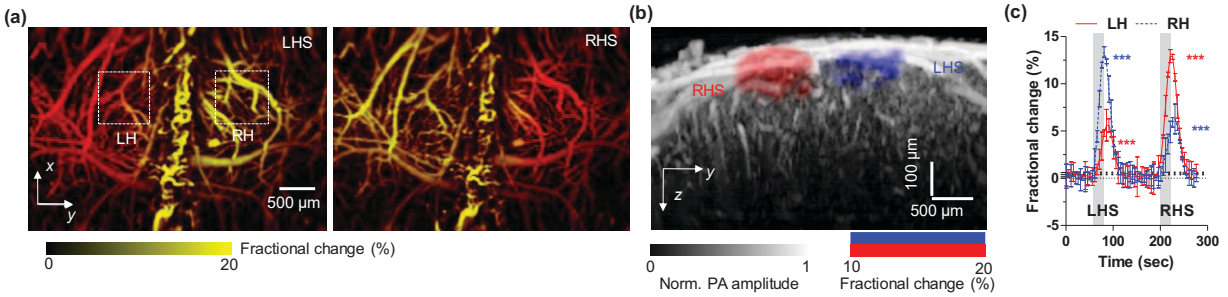

Fig. 2. PAM of brain responses to electrical stimulations of the hindlimbs of mice. (**a**) Fractional photoacoustic amplitude changes (shown in yellow) in response to left hindlimb stimulation (LHS) and right hindlimb stimulation (RHS), superimposed on the vascular image (shown in red). LH/RH, left/right hemisphere. (**b**) Depth-resolved photoacoustic amplitude responses. The responding areas in the LH and RH are shown in red and blue, respectively, and superimposed on the gray-scale *y*-*z* projection image. The signal amplitude in the *y*-*z* projection image was normalized depth-wise. (**c**) Time courses of the fractional changes in the cerebral blood perfusion in the core responding region. Statistics: paired student's *t*-test. *P* values: *** <0.001.

CONCLUSIONS

In summary, using endogenous contrast, PAM can perform high-speed high-resolution imaging of the vascular morphology and blood oxygenation of the mouse brain. In particular, PAM has achieved a 1D time-resolved imaging rate of 500 kHz for morphological imaging and 100 kHz for blood oxygenation imaging. PAM is highly complementary to other brain imaging modalities in its contrast mechanism, spatial–temporal resolutions, and functional imaging capability.

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